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In vitro and *in vivo* antidermatophytic activities of some Iranian medicinal plants

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Abstract

In the last decades, the number of people suffering from dermatophytoses has seriously increased, which may be due to the development of resistant strains to a range of antifungal drugs. The present study was aimed to evaluate the antidermatophytic properties of eight extracts from the selected spices and herbs, which were ethno-medicinally used in Iran against *Trichophyton mentagrophytes, Trichophyton interdigitale*, *Microsporum canis*, and *Microsporum gypseum* (10 strain of each). The *in vitro* antifungal activities of the extracts from four spices and four plants were evaluated by the broth macro dilution method against four dermatophyte strains. In addition, the *in vivo* therapeutic effects of *Myrtus communis* L. and *Cinnamomum zeylanicum* Blume extracts (the most active extracts) on dermatophytosis induced by *M. canis* and *T. mentagrophytes* in guinea pigs were evaluated. Results of *in vitro* antifungal assay revealed that all the tested extracts demonstrated both fungistatic and fungicidal activities with the geometric mean (GM) MIC ranging from 0.058 to 3.73 mg/ml and GM (MFC) ranging from 0.058 to 7.46 mg/ml, respectively. Two extracts (*M. communis* and *C. zeylanicum*) significantly inhibited the growth of all the tested dermatophytes, while other extracts demonstrated weak (MICs of $>$0.625 mg/ml) to moderate (MICs ranging from 100 to 0.625 mg/ml) activities. *In vivo* antidermatophytic assay demonstrated that clotrimazole cured *T. mentagrophytes* and *M. canis* infection on days 21 and 17, respectively, whereas *M. communis* and *C. zeylanicum* extracts significantly ($p < 0.05$) cured *T. mentagrophytes* and *M. canis* infection on days 9 and 13 as well as 9, 11, respectively. Phytochemical screening showed the presence of flavonoids, tannins, phenols, and alkaloids in *M. communis* and alkaloids, flavonoids, and tannins in *C. zeylanicum.* Findings of the present study also provided the scientific evidence that natural plants could be used in traditional medicine for the prevention and treatment of dermatophytic infections.

Key words: Dermatophytes, Antifungal, Spices, Herbs, *Myrtus communis*, *Cinnamomum zeylanicum*.

Introduction

One of the most important groups of fungi causing worldwide human and animal infections is dermatophytes [1]. They are able to invade keratinized tissues, such as hair, skin, and nails to produce an infection dermatophytosis, which is commonly referred to as ringworm. There are different forms of the disease including tinea corporis, tinea pedis, capitis, barbae, cruris, manum, and onychomycosis [1]. The existing treatments for these infections are still limited to a few antifungal agents. However, clinical values of these agents have been limited due to having high toxicity and emergence of drug resistance in their antifungal activities [2,3]. These factors emphasize the urgent need for the development of new effective treatment alternatives. Since the last decades, plant extracts and plant-derived compounds, due to having fewer side effects, low cost, and high availability, have been valuable sources that are commonly used to treat a wide range of disease conditions including infectious diseases [4]. Spices and herbs are a part of the daily food in several parts of the world, comprise the most important products used for flavoring foods and play a major role as the topical or systemic treatment of a wide range of diseases including infectious diseases. Beside their importance for general well-being, they are frequent parts of traditional formulae [5]. Furthermore, the diets rich in bioactive phytochemicals reduce the risk of degenerative disorders such as cancer, diabetes, cardiovascular diseases, and oxidative dysfunction [6–8]. In Iran, use of spices and other aromatic plants as food flavoring has been an integral part of dietary behaviors for centuries. To the best knowledge of the present authors, few studies have investigated the effects of Iranian medical spices and herbs on dermatophytes. Therefore, the present study was designed to evaluate the *in vitro* and *in vivo* antifungal properties of eight extracts from the selected spices and herbs that were ethno-medicinally used in Iran against some pathogenic dermatophyte strains *(Trichophyton mentagrophytes, Trichophyton interdigitale*, *Microsporum canis*, and *Microsporum gypseum)* with high local prevalence in the southeast of Iran.

Material and methods

Chemicals

Crude powder of fluconazole, clotrimazole, and itraconazole as control drugs were obtained from Sigma-Aldrich Chemical Company, GmbH, Riedstr. RPMI-1640 medium with L-glutamine and sabouraud dextrose broth (SDA) were purchased from Sigma-Aldrich, (St. Louis, MO, USA). Also potato dextrose agar (PDA) was prepared from Oxoid, Basingstoke, Hampshire, United Kingdom. All other chemicals and solvents were of analytical grade.

Fungal strains

The strains of *T. mentagrophytes*, *T. interdigitale*, *M. canis,* and *M. gypseum* (10 of each) used in this study were clinical isolates obtained from nail, skin, or hair specimens recovered from patients from Kerman General Hospitals from January to December 2013. The strains were identified using both macroscopic and microscopic conventional standard methods such as slide culture, growth in CMA with 1% glucose, sabouraud dextrose agar with 3% NaCl, hair perforation, growth at 37°C, urease activity, and *Trichophyton* agar. Four standard strains of *T. mentagrophytes* (ATCC 9533), *T. interdigitale* (ATCC 200099), *M. canis* (ATCC 32903), and *M. gypseum* (ATCC 14683), (American Type Culture Collection) were also used for quality control of tests. *T. mentagrophytes* MYA-4439, the dermatophyte quality control strain recommended for use in CLSI document M38-A2, was included on each day of testing. Sabouraud dextrose agar (SDA) was used for the maintenance and culturing of fungal strains. *Trichophyton* strains on the SDA colonies are generally flat and white to cream in color and have a powdery to granular surface, while *Microsporum* strains are glabrous, downy, wooly, or powdery and white to beige or yellow to cinnamon in color.

Collection of plant materials

The four spices and four plants investigated in this work (*Cinnamomum zeylanicum* Blume, *Zingiber officinale* Roscoe, *Heracleum persicum* Boiss, *Elettaria cardamomum* L., *Salvia officinalis* L., *Calendula officinalis* L., *Myrtus communis* L., and *Mentha spicata* L.) were collected from rural regions of Kerman province, southeast of Iran from May to September 2012. They were identified by a botanist of the Botany Department of Shahid Bahonar University, Kerman, Iran. Voucher specimens have been deposited in the Herbarium of Department of Pharmacognosy, School of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran (Table 1).

Preparation of extracts

The powdered air-dried sample (100 g) from each plant was separately extracted by percolation method with methanol (80%) successively for 72 h in room temperature. The extracts were passed through filter paper (Whatman No.3, Sigma, Germany) to remove plant debris. The extracts were finally concentrated in vacuum at 50°C using

Table 1. Characterization of eight medicinal plants of used in the present study.

No.	Scientific Name	Family	Common Name	Part used	Voucher No.
1	*Calendula officinalis* L.	Asteraceae	Marigold	Flower	KF 1367
2	*Cinnamomum zeylanicum* Blume.	Lauraceae	True cinnamon	Bark	KF 1121
3	*Elettaria cardamomum* L.	Zingiberaceae	Green cardamom	Seed	KF 1246
4	*Heracleum persicum* Boiss.	Apiaceae	Persian Hogweed	Leaf, Seed	KF 1143
5	*Mentha spicata* L.	Lamiaceae	Spear mint	Leaf	KF 1420
6	*Myrtus communis* L.	Myrtaceae	Myrtle	Aerial parts	KF 1356
7	*Salvia officinalis* L.	Lamiaceae	Sage	Flower	KF 1432
8	*Zingiber officinale* Roscoe	Zingiberaceae	Ginger	Rhizome	KF 1457

a rotary evaporator (Heidolph, Germany) to remove solvent (methanol) and stored at −20°C, until testing [9].

Phytochemical analysis

The phytochemical analysis of the methanolic extracts was carried out using the following reagents and chemicals: [10] Alkaloids with Mayer and Bushard's reagent, flavonoids by the use of mg and HCl; tannins with 1% gelatin; and 10% NaCl solutions and saponins with the ability to produce suds.

Animals

Inbred male guinea pigs (400 ± 50 g) were bred in Animal House, Faculty of Medicine, Kerman University of Medical Science (Kerman, Iran). They were housed in a colony room with a 12:12 h light, dark cycle at 21 ± 2°C and handled according to the standard protocols for the use of laboratory animals [11]. They were randomly selected and caged individually or in a group, depending on the type of assay, for 5 days prior to the start of the test for acclimatization to the test conditions. The animals were also randomly assigned to control and test groups.

Minimum inhibitory concentration (MIC) determination

The MIC of extracts against tested dermatophytes was determined by broth macrodilution method, according to the protocol M38-A2 of the Clinical and Laboratory Standards Institute (CLSI) for filamentous fungi with some modifications [12]. Prior to testing, dermatophyte strains were subcultured on PDA slants and incubated at 30°C for 7 to 10 days. Mature colonies were covered with 2 ml of sterile physiological saline (0.85%), suspensions were prepared by gently probing the colony with the tip of a sterile Pasteur pipette and transferred to a sterile conical tube, the final volume being adjusted to 5 ml with saline. The resulting mixture of conidia and hyphal was vortex mixed for 15 seconds and the heavy particles allowed to settle for 5–10 minutes. The upper homogeneous suspension was used for further testing. The resulting conidia suspension was counted in a Neubauer chamber and standardized to concentrations of 1×10^5 to 5×10^5 cfu/ml. This suspension was further diluted 1:10 with RPMI-1640 medium broth with L-glutamine and without sodium bicarbonate to final concentrations of 1×10^4 to 5×10^4 cfu/ml. For the broth macrodilution method, 0.9 ml of the final conidia suspensions were mixed with 0.1 ml of the different concentrations of various extracts (0.0625–16 mg/ml) in test tubes and incubated at 30°C for 7 days. The positive control tube contained 0.9 ml of conidial suspension and 0.1 ml of RPMI-1640, and the negative one contained 1 ml of RPMI-1640 only. The minimum concentrations at which no visible growth was observed were defined as the MIC, which were expressed in mg/ml.

Minimum fungicidal concentration (MFC) determination

To determine minimum fungicidal concentration (MFC) values, after reading the corresponding MIC values, 100 μl samples from all optically clear tubes (complete growth inhibition) plus the last tube showing growth were subcultured on SDA Petri dishes. The dishes were incubated at 35°C for a minimum of 3 days, until growth was clearly visible in the control samples, and MFC values were determined as the lowest concentration of extracts for which there was no visible growth.

In vivo antidermatophytic activity

Ethical statement

The experimental procedures carried out in this survey were in compliance with Guidelines of Kerman University of Medical Science (Kerman, Iran) for the care and use of laboratory animals in line with Animal Ethics Committee (83/22).

Dermal infection of animals

In the present study, 55 male guinea pigs were used to evaluate *in vivo* antidermatophytic activity of the extracts. They were randomly assigned to different treatment groups. For each extract, there were four groups, each containing five animals. Three control groups were used; simple control with no infection and no treatment, untreated control receiving distilled water, and positive control group treated with reference antifungal drug clotrimazole at 10 mg/kg of body weight (BW). The hair of an area (5 cm^2) on the back of each guinea pig was shaved, and the skin was slightly scraped by a single use scalpel. Then, 50 μl of the suspension of *T. mentagrophytes* or *M. canis* was inoculated to the surface of about 3 cm^2 area within the shaved zone [13]. Evidence of the infection was revealed by direct observation of the infected area, followed by agar culture of scrapings from the area, and microscopic observation of the resulting fungi from the scrapings.

Treatment of infected animals

Animals were treated by topical application of each extract at concentrations 1 and 2 g/kg BW (selection of these concentrations was based on the primary experiments which also showed that both extracts had no toxicity at these concentrations), started on the sixth day after animal infection and continued daily (each morning) until achieving complete recovery. Effect of the extracts against dermatophytes was evaluated by culturing skin scrapings and hair on SDA for the recovery of viable dermatophytes. These scrapings and hair were collected from the active border of the infection site every two days [14]. The cultures were incubated for 15 days at 28°C. The results were recorded in terms of percentage culture recovery of dermatophytes of the infected site.

$$\%\ \text{Culture recovery} = \frac{\text{Total number of sites showing the presence of dermatophytes}}{\text{Total number of infected sites}} \times 100$$

Statistical analysis

SPSS software (ver. 17, SPSS Inc., Chicago) was used for data entry and statistical analysis. Geometric mean (GM) of MIC and MFC was obtained for all the isolates tested and the differences between the groups were determined using one way analysis of variance (ANOVA). *P*-value of less than .05 was considered to be statistically significant.

Results

Phytochemical analysis

The phytochemical screening of the methanolic extracts showed the presence of flavonoids, tannins, phenols, and alkaloids in *M. communis* and alkaloids, flavonoids, and tannins in *C. zeylanicum*.

In vitro antidermatophytic activity

Results of *in vitro* antifungal assay (GM of MIC and MFC values) are presented in Table 2. The findings indicated that all the extracts demonstrated both fungistatic and fungicidal activities with the GM (MIC) ranging from 0.058 to 3.73 mg/ml and GM (MFC) ranging from 0.058 to 7.46 mg/ml. *M. communis* and *C. zeylanicum* extracts were significantly ($P < .05$) much more effective than the extracts of other plants, fluconazole, and clotrimazole once they exhibited lower MIC and MFC values for all the tested dermatophyte strains, whereas the lowest antidermatophytic effect was related to the extracts of *S. officinalis* and *M. spicata*. Among the tested dermatophytes, *M. canis* was the most sensitive one to the extracts of the selected plants, while *M. gypseum* was less effective. Moreover, clotrimazole, fluconazole, and itraconazole as control drugs exhibited both fungistatic and fungicidal activities with the GM (MIC) ranging from 0.0042 to 0.217 mg/ml and GM (MFC) ranging from 0.128 to 0.238 mg/ml against the tested dermatophytes. However, the difference in antidermatophytic effects between the extracts and the itraconazole was not statistically significant ($P > .05$), whereas it was statistically significant ($P < .05$) between *M. communis* and *C. zeylanicum* extracts and fluconazole. Since the extracts of *M. communis* and *C. zeylanicum* were the most effective ones against the tested dermatophytes on *in vitro* model, the *in vivo* therapeutic effect of these extracts on dermatophytosis induced by *T. mentagrophytes* and *M. canis* in guinea pigs was also evaluated.

In vivo antidermatophytic activity

Figures 1 and 2 demonstrate the *in vivo* efficacy of *M. communis* and *C. zeylanicum* extracts (the most active extracts) for the dermatophytosis induced in guinea pigs. Both extracts were found to be efficacious in a time-dependent manner compared with clotrimazole as the positive control ($P < .05$). *M. communis* with the concentration of 1 g/kg BW resulted in the complete cure of *T. mentagrophytes* and *M. canis* infection on days 11 and 13, respectively; while the concentration of 2 g/kg BW significantly ($p < 0.05$) cured *T. mentagrophytes* and *M. canis* infection on days 9 and 13, respectively. *C. zeylanicum* extract at

Table 2. Geometric mean MIC and MFC of some Iranian medicinal plants against some pathogenic dermathopyte species (10 isolates of each species).

	Fungal strains									
	Trichophyton interdigitale		*Trichophyton mentagrophytes*		*Microsporum canis*		*Microsporum gypseum*		MYA-4439 strain	
Tested samples	MIC (mg/ml)	MFC (mg/ml)	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Salvia officinalis	3.48	6.96	3.73	3.73	3.24	3.73	3.73	3.73	3.24	7.46
Mentha spicata	1.62	3.48	1.51	3.24	3.24	4.0	3.48	7.46	1.62	2.0
Cinnamomum. zeylanicum	0.125	0.122	0.071	0.125	0.0625	0.0625	0.0625	1.51	0.0625	1.62
Calendula officinalis	0.46	0.87	0.57	0.87	0.50	0.50	0.87	2.0	0.46	0.87
Heracleum persicum	0.87	1.86	1.86	1.86	0.87	1.62	3.73	3.73	0.87	1.86
Elettaria camaldulensis	0.87	1.86	0.87	1.86	0.50	0.87	1.62	3.48	0.87	1.86
Myrtus communis	0.099	0.106	0.058	0.106	0.058	0.058	0.099	0.217	0.099	0.217
Zingiber officinale	0.50	0.87	0.43	0.50	0.50	1.0	0.87	0.87	0.43	1.0
Fluconazole	0.147	0.222	0.147	0.238	0.137	0.137	0.137	0.238	0.054	0.128
Itraconazole	0.0091	0.128	0.0091	0.128	0.0042	0.128	0.0042	0.137	0.0091	0.128
Clotrimazole	0.217	0.217	0.153	0.217	0.189	0.189	0.164	0.217	0.189	0.217

Figure 1. Evaluation of the percent culture recovery during treatment of infected guinea pigs with *M. communis* extract: concentration of 1 g/kg BW (a) and concentration of 2 g/kg BW (b).

the concentration of 1 g/kg BW resulted in the complete cure of *T. mentagrophytes* and *M. canis* infection on days 11 and 13, respectively, whereas 2 g/kg BW concentration of this extract significantly ($P < .05$) cured *T. mentagrophytes* and *M. canis* infection on days 9 and 11, respectively. Moreover, the clotrimazole resulted in the complete cure of *T. mentagrophytes* and *M. canis* infection on days 21 and 17, respectively. In the control model, the hair culture was positive, which exhibited 100% culture recovery between days 3 and 21.

Discussion

Historically, herbs and spices have been used as a valuable natural resource for traditional remedy [15]. In recent years, development of adverse effects and microbial resistance to the chemically synthesized drugs has caused changes in the situation and interest in the field of ethnobotanical research [16,17]. Therefore, the present study was aimed to investigate the *in vitro* and *in vivo* antifungal properties of 8 extracts from the spices and herbs ethnomedicinally used in Iran against some pathogenic dermatophyte strains. Results of *in vitro* antifungal assay revealed that all the tested extracts demonstrated both fungistatic

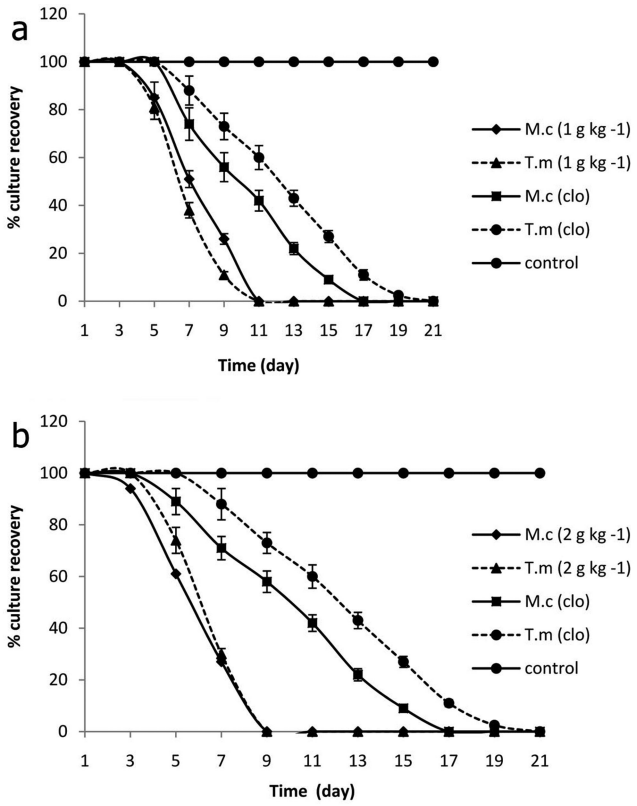

Figure 2. Evaluation of the percent culture recovery during treatment of infected guinea pigs with *C. zeylanicum* extract: concentration of 1 g/kg BW (a) and concentration of 2 g/kg BW (b).

and fungicidal activities with the GM (MIC) ranging from 0.058 to 3.73 mg/ml and GM (MFC) ranging from 0.058 to 7.46 mg/ml. Each of the extracts tested in the present study indicated an antifungal activity on at least one of the tested dermatophytes. However, differences were observed between antifungal activities, since most of the tested plant extracts exerted a broad antifungal spectrum. These variations in antifungal activity could be due to the differences in the chemical composition of these plants, because the secondary metabolites of the plants have many effects including antimicrobial properties [16]. Furthermore, the activity of the plant extracts could be influenced by the nature of the plant material or its origin as well as the climatic conditions in which it grew, the used plant parts, or the solvent used for extraction, because plants have different constituents depending on these factors [18]. Antimicrobial activity of plant extracts is considered to be significant if MIC values are below 100 μg/ml for crude extract and moderate when MICs vary from 100 to 625 μg/ml [18]. In this study, two extracts (25%) significantly inhibited the growth of all the tested dermatophytes, while other extracts demonstrated weak to moderate activities. These findings were in agreement with those of previous studies, reporting that the commonly used herbs and spices had antimicrobial properties that, in some cases, can be used in traditional medicine [19]. Since *M. communis* and *C. zeylanicum* exhibited the best activity against the tested drematophytes in the *in vitro* model, thus the *in vivo* therapeutic effect of these extracts was investigated against dermatophytosis induced in guinea pig model. Dermatophytosis induced in guinea pigs is a well established predictive model for testing topical antifungal agents [13]. This model was used in this study and allowed to indicate that the crude extract of *M. communis* and *C. zeylanicum* had an *in vivo* antifungal activity against some pathogenic strains of dermatophytes. In this model, the significant efficacy of *M. communis* and *C. zeylanicum* extracts, particularly at the concentration of 2 g/kg BW, was observed for dermatophytosis induced in guinea pigs. Skin redness, lesion severity, and dermatophyte occurrence were significantly reduced following the application of the extracts. The efficacy was confirmed by the recurrence of hair growth in the infected areas in treatment models compared with control group. When clotrimazole cured *T. mentagrophytes* and *M. canis* infection on days 21 and 17, respectively, *M. communis* and *C. zeylanicum* extracts significantly ($P < .05$) cured *T. mentagrophytes* and *M. canis* infection on days 9 and 13 as well as 9 and 11, respectively. Therefore, the highest activity of the aforementioned extracts was observed against *T. mentagrophytes* followed by *M. canis* infection. In line with the present findings, several studies have been reported the antidermatophytic action of the 1% oil-petroleum jelly formulation of the essential oil of *Chenopodium ambroisoides* and formulated extract-oil (5%) of *Polyscias fulva* curing *T. mentagrophytes*-induced dermatophytosis in guinea pigs on days 15 and 14, respectively [20,21]. In addition, similar efficacies have been proven on the extract-cream formulation of *Zataria multiflora* and *Eucalyptus camaldulensis* [22,23].

Various pharmacological activities such as antioxidant, anti-inflammatory, anticancer, and antimicrobial effects have been related to *M. communis* and *C. zeylanicum* [24,25]. In addition, phytochemical screening of the crude extract of *M. communis* and *C. zeylanicum* has revealed the presence of tannins, alkaloids, flavonoids, and phenols in these plants. Individual activities of these compounds have also been proven [16]. Various studies have reported the potent antifungal effects of these compounds and their derivatives such as thymol and carvacrole against some pathogenic fungal strains [26–28]. Carvacrol was active *in vitro* against 100 clinical isolates of *Candida albicans* with MICs ranging from 0.125 to 0.004%. MIC (50) and MIC (90) values of carvacrol were observed at 0.064 and 0.125 mg/ml, respectively [26]. In addition, thymol was more effective against *T. rubrum* with MIC and MFC values of 11.7 to 23.4 μg/ml, respectively [27].

Therefore, phytoconstituents in these plants could be responsible for their antidernatophytic activity although their exact mode of action is poorly understood. However, it has been previously shown that antimicrobial effects of carvacrol and thymol are attributed to their ability to permeabilize and depolarize the cytoplasmic membrane [29]. In dermatophytic infections, inflammatory responses are usually defined by a greater degree of redness and scaling at the edges of the lesions, or occasionally, blister formations [30]. During the inflammation, reactive oxygen species and free radicals with many physiological and pharmacological adverse effects, including skin irritation, are produced [31]. However, some compounds of *M. communis* and *C. zeylanicum* extracts such as phenols and tannins are known for their ability in protecting cells against reactive oxygen species and free radical-induced toxicity [32].

In conclusion, this study demonstrated the antidermatophytic effects of *M. communis* and *C. zeylanicum* extracts. Findings of the present study also provided the scientific evidence that natural plants could be used in traditional medicine for the prevention and treatment of dermatophytic infections.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References

1. Weitzman I, Summerbell RC. The dermatophytes. *Clin Microb Rev* 2001; **8**: 240–259.
2. Watanabe S. Present state and future direction of topical antifungals. *Japaneses J Med Mycol* 1990; **40**: 151–155.
3. De Pauw BE. Is there a need for new antifungal agents? *Clin Microbiol Infect* 2000; **6**: 23–28.
4. Rocha LG, Almeida JR, Macedo RO et al. A review of natural products with antileishmanial activity. *Phytomedicin* 2005; **12**: 514–535.
5. Mandeel QA, Al-Laith AA. Ethnomycological aspects of the desert truffle among native Bahraini and non-Bahraini peoples of the Kingdom of Bahrain. *J Ethnopharmacol* 2007; **110**: 118–129.
6. Bazzano LA, Serdula MK, Liu S. Dietary intake of fruits and vegetables and risk of cardiovascular disease. *Curr Athero Rep* 2003; **5**: 492–499.
7. Sherry E, Sivananthan S, Warnke PH et al. Topical phytochemicals used to salvage the gangrenous lower limbs of type 1 diabetic patients. *Diabetes Res Clin Prac* 2003; **62**: 65–66.
8. Lee KW, Lee HJ, Lee CY. Vitamins, phytochemicals, diets, and their implementation in cancer chemoprevention. *Crit Rev Food Sci Nutr* 2004; **44**: 437–452.
9. Mahmoudvand H, Sepahvand A, Jahanbakhsh S et al. Evaluation of antifungal activities of the essential oil and various extracts of *Nigella sativa* and its main component, thymoquinone against pathogenic dermatophyte strains. *J Mycol Med* 2014; **24**: 155–161.
10. Evans WC. *Trease and Evans Pharmacognosy*. 14th edition, WB Saunders Company Limited, 1998; pp. 15–16.
11. OECD guidelines for testing of chemicals. Repeated dose dermal toxicity 21/28-day study. *OECD guideline for testing chemicals* 1981; **410**: 1–8.
12. CLSI/Clinical and Laboratory Standards Institute, 2002. *Reference method for broth dilution antifungal susceptibility testing of conidium-forming filamentous fungi*. Wayne. (Approved Standard M38-A).
13. Polak A. Experimental models in antifungal chemotherapy. *Mycoses* 1998; **41**: 1–30.
14. Cray SM. *Douglas: Mycology for clinical laboratories*. 1997, Virginia: Reston publishing.
15. Jones FA. Herbs and useful plants. Their role in history and today. *European J Gastroenterol and Hepatol* 1996; **8**: 1227–1231.
16. Cowan MM. Plant products as antimicrobial agents. *Clin Microbiol Rev* 1999; **12**: 564–582.
17. McCutcheon AR, Ellis SM, Hancock REW et al. Antibiotic screening of medicinal plants of the British Columbian native peoples. *J Ethnopharmacol* 1992; **37**: 213–223.
18. Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *Africa J Biotech* 2008; **7**: 1797–1806.
19. Lai PK, Roy J. Antimicrobial and chemopreventive properties of herbs and spices. *Curr Med Chem* 2004; **11**: 1451–1460.
20. Aghel N, Moghimipour E, Ameri A. Characterization of an antidermatophytic cream from *Zataria multiflora* Boiss. *Iran J Pharm Sci* 2007; **3**:77–84.
21. Njateng GS, Gatsing D, Mouokeu RS et al. *In vitro* and *in vivo* antidermatophytic activity of the dichloromethane-methanol (1:1 v/v) extract from the stem bark of *Polyscias fulva* Hiern (Araliaceae). *BMC Complement Altern Med* 2013; **6** (13): 95.
22. Moghimipour E, Ameri A, Saudatzadeh A et al. Formulation of an antidermatophytic cream from hydro-alcoholic extract of *Eucalyptus camaldulensis* leaves. *Jundishapur J Nat Pharm Prod* 2009; **4**: 32–40.
23. Thirumalai T, David E, Viviyan SV et al. Restorative effect of *Eclipta alba* in CCl4 induced hepatotoxicity in male albino rats. *Asian Pac J Trop Med* 2011; 304–307.
24. Sharififar F, Moshafi MH, Dehghan-Nudehe G et al. Bioassay screening of the essential oil and various extracts from 4 spices medicinal plants. *Pakistan J Pharm Sci* 2009; **22**: 317–322.
25. Alipour G, Dashti S, Hosseinzadeh H. Review of Pharmacological Effects of *Myrtus communis* L. and its Active Constituents. *Phytother Res* 2014; **28**: 1125–1136.

26. Vardar-Unlu G, Yağmuroğlu A, Unlu M. Evaluation of *in vitro* activity of carvacrol against *Candida albicans* strains. *Nat Pro Res* 2010; **24**: 1189–1193.
27. de Melo JO, Bitencourt TA, Fachin AL et al. Antidermatophytic and antileishmanial activities of essential oils from *Lippia gracilis* Schauer genotypes. *Acta Trop* 2013; **128**: 110–115.
28. Abbaszadeh S, Sharifzadeh A, Shokri H et al. Antifungal efficacy of thymol, carvacrol, eugenol and menthol as alternative agents to control the growth of food-relevant fungi. *J Mycol Med* 2014; **24**: 51–56.
29. Xu J, Zhou F, Ji BP et al. The antibacterial mechanism of carvacrol and thymol against *Escherichia coli*. *Lett Appl Microbiol* 2008; **47**: 174–179.
30. Hainer BL. Dermatophyte Infections. *American Fam Phys* 2003; **67**: 101–109.
31. Kishore N, Chansouria JPN, Dubey NK. Antidermatophytic action of the essential oil of *Chenopodium ambrosioides* and an ointment prepared from it. *Phytother Res* 1996; **10**: 453–455.
32. Zielinska M, Kostrzewa A, Ignatowicz E et al. The flavonoids, quercetin and isorhamnetin 3-O-acylglucosides diminish neutrophil oxidative metabolism and lipid peroxidation. *Acta Bioch Pol* 2001; **48**: 183–189.